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Studies with Temocillin in a Hamster Model of Antibiotic-Associated Colitis

RONALD J. BOON* AND ANGELA S. BEALE

Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, England

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Hamsters given the new penicillin temocillin, either orally or by injection, did not develop antibiotic-associated colitis, whereas animals given the control antibiotics cefoxitin or clindamycin developed the disease, which is characterized by marked hemorrhagic cecitis and high cecal levels of *Clostridium difficile* cytotoxin.

Antibiotic-associated colitis (AAC) is one of the most severe reactions to antimicrobial drug administration and can be fatal if left untreated. Antibiotic administration is believed to produce an imbalance in the gut flora, allowing acquisition and proliferation of cytotoxin-producing strains of *Clostridium difficile* (8). Considerable advances in the understanding of AAC have been made with the use of animal models of colitis. Most antibiotics which produce AAC in humans also produce the disease in hamsters. The hamster model is therefore regarded as a sensitive indicator of antibiotics likely to cause AAC in treated patients (5).

Temocillin is a new β-lactamase-stable penicillin which has a defined spectrum of activity, being active against most members of the *Enterobacteriaceae*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. It is not, however, active against gram-positive bacteria, *Pseudomonas aeruginosa*, and anaerobes, including *C. difficile* (6). The aim of the studies described here was to ascertain whether temocillin would produce AAC in the hamster model. Clindamycin and cefoxitin were used as control antibiotics, because these agents have been well documented as being capable of causing AAC in this model (1, 3).

Three dosing regimens were used, with the antibiotics being tested concurrently in each regimen. (i) A single dose of the antibiotic was administered subcutaneously (s.c.) to groups of five Syrian hamsters. Temocillin (Beecham Pharmaceuticals) or cefoxitin (Merck Sharp & Dohme) was administered at 1, 10, or 100 mg per hamster and clindamycin (The Upjohn Co.) was administered at 1 mg per hamster in 0.5 ml of phosphate-buffered saline. Control animals received phosphate-buffered saline. (ii) Doses (1 and 10 mg) of temocillin or cefoxitin were administered s.c. three times daily for 4 days to determine the effect of multiple dosing. (iii) Hamsters received a single oral dose (p.o.) of clindamycin (1 mg), temocillin (10 mg), or cefoxitin (10 mg). These doses were selected because of their performances in a previously reported study (3). The hamsters were weighed daily for 10 days, and their cages and bedding were inspected for signs of diarrhea. The gross pathology of the intestine was examined when the animals died or at the end of the experiment, and time to death for 50% of the population (TD_{50}) was calculated. Histology was done on the ceca, and the cecal contents were collected for tissue culture toxin assay with mouse fibroblast cells, according to the method of Borriello (2).

Hamsters treated p.o. or s.c. with temocillin gained weight at a rate similar to that of saline-dosed control animals, about 14 g in a 10-day period. Cefoxitin administered by either route induced a rapid weight loss, about 10 g in 4 days, in animals that succumbed to AAC. Clindamycin-treated hamsters showed a small weight gain, but all died within 6 days.

The data in Table 1 illustrate the effects of single s.c. doses of clindamycin, cefoxitin, temocillin, or saline. The TD_{50} for hamsters dosed with 1 mg of clindamycin was 5 days, all animals had diarrhea. On postmortem, marked hemorrhage and distension of the cecum and also of the ileum and colon were noted. Cecal filtrates from all the clindamycin-treated hamsters were cytotoxic at a 10^{-4} dilution and were neutralized by *Clostridium sordellii* antitoxin, indicative of high *C. difficile* cytotoxin levels (1). Of the hamsters dosed s.c. with 1 mg of cefoxitin, 60% (3 of 5) were alive at the termination of the experiment (TD_{50} >10 days) and showed no evidence of AAC at postmortem. The remaining animals in this group had diarrhea before death, and high toxin levels were present in their cecal filtrates despite the fact that no gross pathological abnormalities were observed.

The incidence of AAC was greater with both the 10- and 100-mg doses of cefoxitin, with all the animals dying. The TD_{50}s were reduced to 3 and 6 days for the 10- and 100-mg doses, respectively. Before death 80 to 100% of the animals had diarrhea, 60 to 80% had hemorrhagic cecitis on postmortem, and 100% had high *C. difficile* toxin levels in the cecal filtrates. In contrast, none of the animals that received temocillin or saline died. The pathological features indicative of AAC were absent at postmortem, and the cecal filtrates from these animals were all toxin negative. Animals administered temocillin at 1 and 10 mg s.c. three times daily for 4 days showed no difference in response to that observed after a single dose. With multiple dosing of cefoxitin at 1 mg there was an increased incidence of AAC compared with the single dose, whereas the 10-mg multiple dose resulted in a delayed onset of AAC.

Histopathological study of the ceca showed that in animals administered a single s.c. dose of clindamycin, marked hemorrhagic congestion and necrosis of the entire mucosa occurred in some cases, and areas of erosion to the full depth of the mucosa were observed in others. An acute inflammatory reaction was evident in all sections. Cefoxitin produced a similar response, with marked necrosis and sloughing of the entire superficial and glandular epithelium. With temocillin dosage of 1, 10, or 100 mg, there was only a slight infiltration of polymorphonuclear leukocytes into the lamina propria, comparable to that in the saline controls. These changes were regarded as being within normal limits.

* Corresponding author.

TABLE 1. Assessment of AAC in hamsters after single or multiple s.c. doses or a single p.o. dose of temocillin, cefoxitin, and clindamycin

Dosage regimen			No. of fatalities/ no. treated (%)	TD_{50} in days (range)	% Hamsters with:	
Compound	Dose (mg/kg) × frequency	Route			Diarrhea and/or hemorrhage and cecal distension	Toxin-positive filtrates
Temocillin	1 × 1	s.c.	0/5 (0)	>10	0	0
	10 × 1	s.c.	0/5 (0)	>10	0	0
	100 × 1	s.c.	0/5 (0)	>10	0	0
	1 × 3[4a]	s.c.	0/5 (0)	>10	0	0
	10 × 3[4]	s.c.	0/5 (0)	>20	0	0
Cefoxitin	1 × 1	s.c.	2/5 (40)	>10 (2->10)	40	40
	10 × 1	s.c.	5/5 (100)	3 (2–4)	100	100
	100 × 1	s.c.	5/5 (100)	6 (3–7)	80	100
	1 × 3[4]	s.c.	5/5 (100)	6 (5–13)	100	100
	10 × 3[4]	s.c.	4/5 (80)	13 (7->20)	80	80
Clindamycin	1 × 1	s.c.	5/5 (100)	5 (4–6)	100	100
Saline		s.c.	0/5 (0)	>10	0	0
Temocillin	10 × 1	p.o.	0/5 (0)	>20	0	0
Cefoxitin	10 × 1	p.o.	5/5 (100)	4 (4–5)	100	100
Clindamycin	1 × 1	p.o.	4/5 (80)	6 (5->20)	80	80
Saline		p.o.	0/5 (0)	>20	0	0

[a] Compounds given three times a day for 4 consecutive days.

When hamsters received a single p.o. dose of 1 mg of clindamycin or 10 mg of cefoxitin, the severity of the AAC induced was similar to that following the s.c. dose (Table 1). The TD_{50} were 6 and 4 days, respectively, for clindamycin and cefoxitin, and the majority of hamsters had diarrhea, hemorrhagic cecitis, and high cecal *C. difficile* cytotoxin levels. Again, none of the p.o. temocillin-treated hamsters developed AAC.

The importance of the anaerobic fecal flora in maintaining resistance to infection by exogenous potential pathogens is well established (7). Studies in mice and in humans (H. G. DeVries-Hospers, W. Hofstra, and G. W. Welling, Program Abstr. 22nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 771, 1982) have shown that after temocillin therapy there is no disturbance of the anaerobic fecal flora, whereas the *Enterobacteriaceae* are eliminated from the flora. The absence of antibiotic-associated colitis in hamsters after temocillin treatment therefore may result from the lack of activity of temocillin against the anaerobic flora, thereby maintaining resistance to colonization by exogenous *C. difficile* (4).

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